# BIOCONVERSION OF AGRICULTURAL SOLID WASTE (BANANA TREE STEM) BY MIXED CULTURE FOR USE AS CARBON SOURCE FOR FERMENTATION MEDIUM

**MASHAIDA BINTI MD SHARIF**

**A thesis submitted in fulfillment**
**of the requirements for the award of the degree of**
**Bachelor of Chemical Engineering (Biotechnology)**

**Faculty of Chemical & Natural Resources Engineering**
**Universiti Malaysia Pahang**

**April 2009**

I declare that this thesis entitled “Bioconversion of Agricultural Solid Waste (Banana Tree Stem) By Mixed Culture for Use as Carbon Source for Fermentation Medium” is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.”

Signature :……

Name : Mashaida Binti Md Sharif

Date : 30 April 2009

*“Specially dedicated to my beloved father, mother and sisters”*

## ACKNOWLEDGEMENT

I would like to express my deepest and warm gratitude to all of the following persons for their helps and kindness and also their guidance which had enabled me to complete my Undergraduate Research Project in time for the fulfillment of the requirements of the degree of Bachelor of Chemical Engineering (Biotechnology).

First and foremost, I would like to thank my supervisor, Prof Ir. Dr. Jailani Bin Salihon and my co-supervisor, Madam Nor Azwina Binti Zainol Abidin who had helped me a lot in the progress of my projects by giving me the required information and also the valuable opinions. Also, thanks for sparing me your precious time in assisting me in my project and also answering to all of my doubts and confusions.

Also, I would like to forward my appreciation to the panels, Miss Nasratun Binti Masngut and Mr. Rozaimi bin Abu Samah for the guidance and also the suggestions. To all the technical staffs of Chemical Engineering Lab, a big thanks for all of you for assisting me in my experimental setups and also in the progress of my experiment at the laboratory.

For all of my family members, father, mother, Masnita, Maslina, Masmira, Maslela Ayu, Mas Erma and Mastikaratna, thanks for all the support and encouragement. You had convinced me that success can be achieved with hard work, patience and enthusiasms. And last but not least, a very special thanks to my friends and colleagues who had helped me either directly or indirectly upon completing my projects.

# ABSTRACT

Lignocellulosic waste can be useful if the cellulose can be freed from the lignin so that the cellulose can be converted into sugar and be used as the carbon source for fermentation medium. The glucose can be produced by anaerobic digestion which involve the process of breaking up the lignin components or delignification and later on followed by cellulose degradation. This thesis reports on the research in the delignification of lignocellulose in banana stem waste and the degradation of the cellulose into glucose. The mixed culture was collected from a banana plantation field. The process is carried out at ambient temperature in two anaerobic digesters with different organic loading rates and also with and without acclimatization respectively. From the result, it is found that glucose is recovered at the end of the digestion process but having different conversion with the anaerobic digester having the higher loading rates and acclimatized culture giving higher conversion. The percentage of lignin loss due to the delignification that occurred is determined by analyzing the sample using Klason (72% sulphuric acid) Method. The recovered glucose from the anaerobic digestion is tested as fermentation medium for *Saccharomyces cerevisiae* (yeast) and the results had shown that the yeast is capable of utilizing the glucose as carbon source. Hence, it can be concluded that the banana stem waste can be bioconverted into carbon source for fermentation medium.

# ABSTRAK

Bahan buangan berlignoselulosa boleh mendatangkan manfaat apabila selulosa dapat dipisahkan daripada lignin dan seterusnya dijadikan sebagai sumber karbon bagi medium untuk fermentasi. Glukos dapat dihasilkan sebagai sumber karbon dalam proses fermentasi melalui pencerna anaerobik yang melibatkan proses pemecahan lignin dan seterusnya diikuti oleh proses penguraian selulosa. Tesis ini membincangkan proses penguraian lignoselulosa kepada glukos daripada bahan buangan batang pisang menggunakan kultur campuran yang dikumpulkan dari kebun pisang. Proses ini dijalankan pada suhu persekitaran di dalam dua pencerna anaerobik berlainan yang mempunyai kadar muatan bahan organik yang berbeza serta dilakukan proses penyesuaian dan tidak disertai oleh proses penyesuaian. Daripada keputusan kajian, didapati bahawa glukos terhasil pada akhir proses pencernaan anaerobik namun pada kadar pertukaran yang berbeza di mana kadar pemerolehan glukos adalah lebih tinggi di dalam pencerna anaerobik yang mempunyai kadar muatan bahan organik yang lebih tinggi dan disertai penyesuaian terlebih dahulu. Kemudian, kadar peratusan kehilangan lignin turut ditentukan menerusi kaedah Klason (72% asid sulfurik). Akhir sekali, glukos yang telah terhasil diuji sebagai medium fermentasi bagi *Saccharomyces cerevisiae* (yis) dan melalui pemerhatian yang dibuat, yis dapat menggunakan glukos tersebut sebagai sumber karbon. Justeru, dapat disimpulkan bahawa bahan buangan batang pisang dapat ditukarkan secara biologi kepada sumber karbon bagi proses fermentasi secara pencernaan anaerobik mengunaakan kultur campuran.

# TABLE OF CONTENTS

# LIST OF FIGURES

## LIST OF SYMBOLS/ABBREVIATIONS

| | | |
|---|---|---|
| DNS | - | Di-Nitro Salicylic Acid |
| Glu. | - | Glucose |
| g | - | gram |
| h | - | hour |
| mg/L | - | milligram per liter |
| Min | - | minutes |
| mL | - | mililiter |
| v/v | - | volume per volume |
| v/w | - | volume per weight |
| w/v | - | weight per volume |
| w/w | - | weight per weight |
| g/L | - | gram per liter |
| % | - | percentage |
| °C | - | degree Celsius |

## LIST OF APPENDICES

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Study

The bio-based chemical industry is growing rapidly day by day. Over the long term, the requirement for the fermentable sugars as the feedstock to support the bio-based chemical industry will be very high to meet the demand of the industry. The existing commercial microbial fermentation is utilizing the glucose to produce the ethanol, acetic acid, amino acids, antibiotics and also other chemicals. The future of fermentation technology will be greatly enhanced by lignocelluloses conversion (Dale, 1987). The fermentable sugars can be found in agricultural waste and also in crops and the sugars can be produced either directly or is derived from the polysaccharides such as the cellulose and hemicelluloses. Cellulose and hemicelluloses are carbohydrates that can be broken down by enzymes, acids, or other compounds to simple sugars, and then fermented to produce other product such as the renewable electricity, fuels, and biomass based products (Puri, 1984; Wyman and Goodman, 1993; van Wyk, 2001)

The banana stem waste has a high organic content (83%); with 15±20% (w/w) lignin and cellulose which gives it a sheath-like texture (Kalia *et al*., 1999). In tropical and subtropical areas, the banana is produced in large quantities. The total planted area of banana in Malaysia in 2001 was estimated to be 33,704.2 hectares (MAO, 2006). Normally, after the harvesting of the banana fruits, the whole plant which are the stem,

leaves and also the rhizome is left at the plantation field for natural degradation and this process will take several months. However, these banana wastes can be utilized effectively for the releasing of sugars for carbon source which can later on be used as fermentation medium (Khalil *et al,*. 2006).

Before the desired carbon source which is glucose can be obtained from the banana stem waste, a sequence of procedures should be done first. The process is the degradation of the lignin (delignification) and also the degradation of polysaccharide (cellulose degradation) which are the components of the lignocelluloses.

## 1.2 Objective

The aim of this study/research is to biologically convert the agricultural solid waste (banana tree stem) to carbon source for fermentation medium. Also, this study is done to investigate the delignification of banana stem waste by mixed culture. Lastly, it is also the aim of this study to produce the carbon source from the cheap source (banana stem waste).

## 1.3 Scope of Study

The scope of doing this study is to investigate the anaerobic digestion of the lignin and polysaccharide components of the banana stem waste which will further be converted into the carbon source for the fermentation medium. In converting the agricultural solid waste as the carbon source for the fermentation medium, several processes will be conducted. First of all is the process of acclimatizing the anaerobic microflora from the soil which is taken from the banana plantations at ambient temperature which is the best temperature for mixed culture from garden soil (Benner *et al,*. 1984) and also the

optimum temperature for lignin degradation (Bumpus and Aus, 1985;Janshekar and Fiechter, 1988). After several months of acclimatization, the sample is transferred into the anaerobic digester to be degraded. The products of the anaerobic decomposition are then being analyzed using the DNS method for glucose and also using the Klason method for lignin constituents.

## 1.4 Problems Statement

The banana stem is one of the agricultural solid wastes which comprises of the lignocellulosic agricultural waste. The waste can be useful if the cellulose can be freed from the lignin so that the cellulose can be converted into sugar and be used as the compound for fermentation medium. The cellulose from the lignocellulosic waste can be hydrolyzed by acid to glucose, but much of the glucose will be destroyed during the process. Also, enzymatic hydrolysis using mixtures of enzymes, such as cellulase and hemicellulaces can be used to avoid the destruction of sugars associated with acid treatments (hydrolysis) of lignocellulosic material. These enzymes will provide high yields of glucose and also other fermentable sugars with minimal sugar losses when combined with effective pretreatment of lignocellulosics. However, these enzymes are currently too costly to be used in large scale conversion of lignocellulosic materials to fermentation substrates. The cost of carbohydrate raw material influences the economy of many fermentation processes, hence the cost play a decisive role in future and scope of industries employing fermentation processes (Dale, 1987; Castellanos *et al*., 1995). Therefore, the use of mix culture from the soil can be a new alternative to produce the glucose for fermentation medium via the anaerobic digestion of the lignocellulosic substances which is not only cheap but in turn will also utilize the waste to be converted into useful products.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Bioconversion

The bioconversion is the biological processes for the conversion of wastes to fuels include ethanol fermentation by yeast or bacteria, and methane production by microbial consortia under anaerobic conditions. Bioconversion is referred to as the enzyme-mediated conversion of organic substrates, such as cellulose, to other more valuable substances, such as protein, and also sugars by other organisms. The conversion of biomass to useable energy, as by burning solid fuel for heat, by fermenting plant matter to produce fuel, as ethanol, or by bacterial decomposition of organic waste to produce methanol is also referred to as bioconversion (Okonko *et al*., 2006).

## 2.2 Banana

Banana (*Musacea sp*.) which is a herbaceous monocots grows in large quantity in both the tropical and also the subtropical area. In 2001, there was approximately 33,704.2 hectares of the total planted area of banana in Malaysia (MAO, 2006). Banana plants range in height from 0.8m to more than 15m. Each contains the flattened and modified stem which is called the ‘pseudostem’. This ‘pseudostem’ consists of concentric layers of leaf sheath and column of large leaves (Ennos *et al.,* 2000). The leaves, which are among

the largest of all the banana plants, can become up to 9 ft long and 2 ft wide. After the banana fruit is harvested, the ‘pseudostem’ is usually being left and wasted. According to N. Saha and G.P. Nagori, banana stem contains 22% lignin and 35% cellulose on dry weight basis.

## 2.3 Lignocellulose

Lignocellulose is mainly composed of cellulose, hemicellulose, and lignin (Sjöström, 1993). Fengel and Wegener , (1989) and Argyropoulos and Menachem , (1997) estimate that there is 2.5−4×1011 tons of cellulose and 2−3×1011 tons of lignin in the earth, representing 40% and 30% of organic matter carbon, respectively, with other polysaccharides comprising 26%. A variety of fungi and bacteria can break down the lignocellulose using hydrolytic and oxidative enzymes (Erikson *et al*., 1999) Photosynthesis and degradation of lignocellulose are essential for the global carbon cycle (Brown 1985, Colberg 1988). The degradation rate is governed by temperature, moisture content, and type of lignocellulose (Rayner and Boddy , 1988, Kuhad *et al*., 1997). A warm, wet environment in contrast to a cold, dry one enhances degradation (Rayner and Boddy , 1988), and herbaceous litter degrades considerably faster than wood (Kuhad *et al*.,1997).

## 2.4 Lignin

Lignin is a natural composite material in all vascular plants, providing the plant with strength and ridigity (Brown, 1985).It is the third most abundant natural polymer present in nature after cellulose and hemicelluloses. The estimated amount of lignin on earth is 300 billion metric tonnes with an annual biosynthetic production rate of 20 billion metric tones (Argyropoulos and Menachem, 1998).Lignin is a complex polymer of phenylpropane units, which are cross-linked to each other with a variety of different

chemical bonds. This complexity has thus far proven as resistant to detailed biochemical characterization as it is to microbial degradation, which greatly impedes our understanding of its effects. Nonetheless, some organisms, particularly fungi, have developed the necessary enzymes to break lignin apart. The initial reactions are mediated by extracellular lignin and manganese peroxidases, primarily produced by white-rot fungi (Kirk and Farrel, 1987). Actinomycetes can also decompose lignin, but typically degrade less than 20 percent of the total lignin presents (Crawford, 1986; Basaglia *et al*., 1992). Lignin degradation is primarily an aerobic process, and in an anaerobic environment lignin can persist for very long periods (Van Soest ,1994). The enzymatic equipment for depolymerizing lignin can be found in fungi and bacteria. Several types of enzymes involved in degradation have been described (Kirk & Farrell, 1987). These include monooxygenases (phenoloxidases, laccases), dioxygenases and peroxidases.

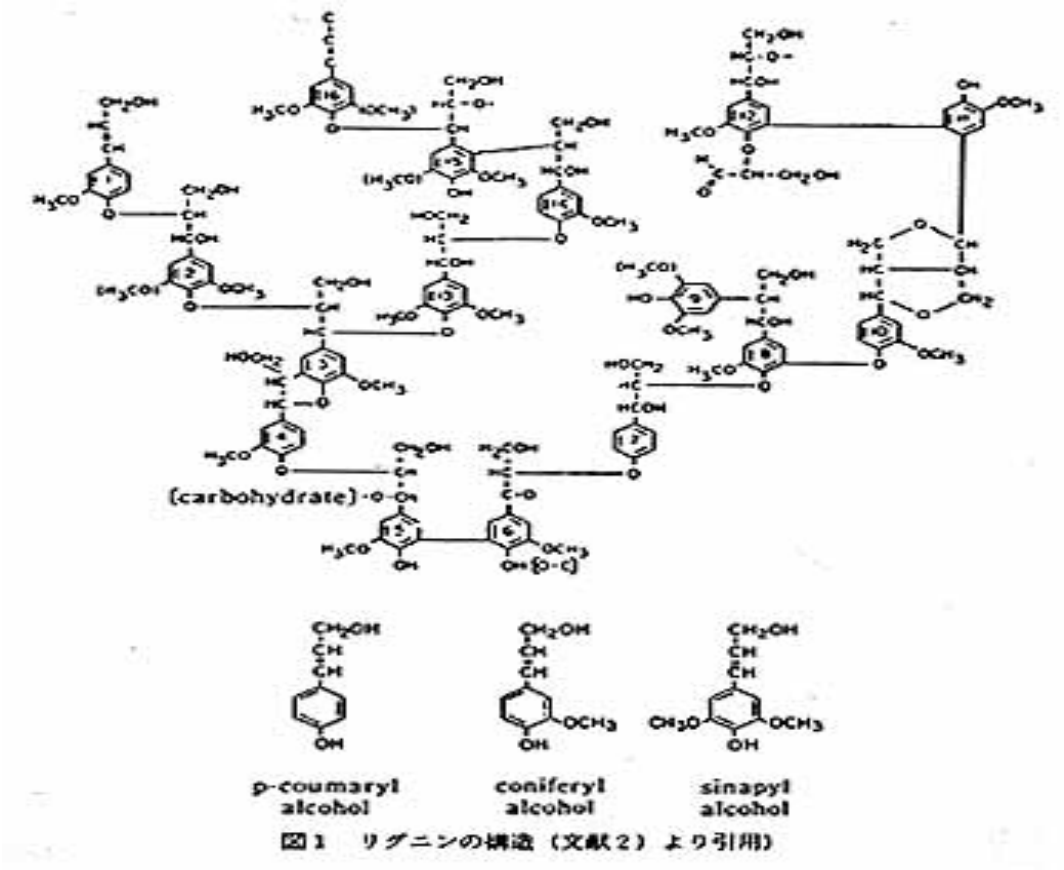


**Figure 2.1**: Lignin Structure

## 2.5 Cellulose

Cellulose is the main constituents of plant cell walls comprising about 50% of wood. Cellulose is a long chain of glucose molecules, linked to one another primarily with β,1-4 glycosidic bonds. The simplicity of the cellulosic structure, using repeated identical bonds, means that only a small number of enzymes are required to degrade this material. Cows and other ruminants create an environment in their rumen which encourages the microbial degradation, converting cellulose to volatile fatty acids and microbial biomass which the ruminant can then digest and use.

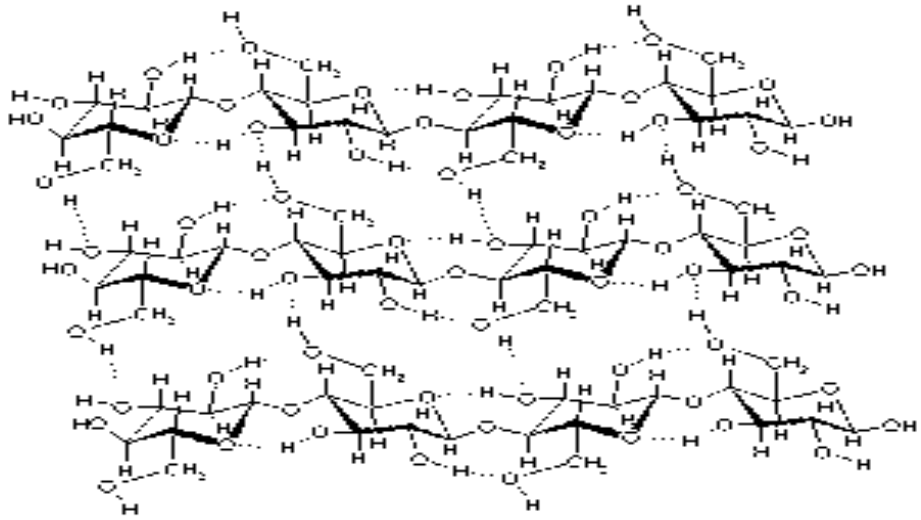

**Figure 2.2**: Cellulose Structure

## 2.6 Hemicelluloses

Hemicelluloses are branched polymers of xylose, arabinose, galactose, mannose, and glucose. Hemicelluloses bind bundles of cellulose fibrils to form microfibrils, which enhance the stability of the cell wall. They also cross-link with lignin, creating a complex web of bonds which provide structural strength, but also challenge microbial degradation (Ladisch *et al*, 1983; Lynch, 1992)

## 2.7 Lignin-degrading microorganisms

### 2.7.1. Aerobic bacteria

Pure bacterial cultures are unable to perform efficient lignin degradation (Blanchette, 1995; Daniel and Nilsson, 1998). The lignin degradation mechanism of bacteria is more specific than that of fungi in which one bacterial species is able to cleave only one type of bond in the lignin polymer (Vicuña *et al.,* 1993). Thus, bacteria degrade lignocellulose in mixed cultures, either in mixed bacterial cultures, or more commonly, in bacterial and fungal cultures together (Vicuña *et al*., 1993; Daniel and Nilsson, 1998). Actinomycetes live in environments rich in lignocellulose, such as soil, compost, heaps of hay, straw, or wood chips (Lacey 1988). Actinomycetes frequently degrade, modify, or solubilize lignin polymer, especially lignin of gramineous plants, to acid precipitable polymeric lignin (Crawford et al. 1983, Adhi *et al*., 1989, Ball *et al*., 1989, Pasti *et al*., 1991, Spiker *et al*. 1992). While degrading the lignocellulosic complex, actinomycetes may also polymerize lignin fragments (Crawford, 1988) or mineralize lignin to some extent (Haider and Trojanowski ,1980). Although lignin mineralization by actinomycetes is not as efficient as by fungi, it is still more efficient than by unicellular bacteria.

### 2.7.2 Anaerobic conditions

In nature, most lignocellulose is degraded by aerobic microorganisms, but a substantial amount is also degraded under anaerobic conditions, such as in soil and compost microenvironments (Atkinson *et al*. 1996, Durrant, 1996). Hackett *et al*., (1977) and Odier and Monties (1983) observed no degradation of lignin in anaerobic conditions. However, in the study of Benner and Hodson (1985), a mixed population isolated from compost mineralized 2-4% of lignin and 14-22% of Kraft lignin under anaerobic conditions at 55°C. According to Colberg and Young (1985), a mixed population isolated

from activated sludge was able to cleave the β-O-4 linkage of low molecular mass lignin in anaerobic conditions, producing monoaromatic compounds. Mineralization of lignin was 6% (Colberg and Young, 1985). In rumen, up to 50% of lignin is either solubilized or transformed into a soluble lignincarbohydrate- complex and a variable amount is digested, although the biochemical pathways are unknown (Susmel and Stefanon ,1993).

### 2.7.3 Mixed populations

The lignin degradation studies of pure microbial cultures have a limited value in understanding the process of mixed populations in soil and in compost because the complex populations involve several interactions that may either stimulate or inhibit the lignin-degrading organisms (Rayner and Boddy ,1988, Carlile and Watkinson , 1994). In forest soil, lignocellulose and lignin are mainly degraded by basidiomycetous litter decomposing fungi, whereas in arable soil and in compost, microfungi are mostly responsible for the lignin degradation since Basidiomycotina are not able to compete with other organisms in these environments (von Klopotek, 1962; Brown 1985). In wood chip or sawdust piles, thermophilic microfungi and bacteria usually dominate, but white rot fungi, such as *Phanerochaete chrysosporium* and *Pleurotus ostreatus*, have been found as well (Rayner and Boddy , 1988; Eaton and Hale ,1993). In mixed populations of soils and composts, actinomycetes are important for lignin degradation, especially at temperatures too high for fungi, but also because they stimulate lignin degradation of some fungi (Waksman and Cordon 1939, Waksman *et al*. 1939a, 1939b, Crawford ,1988, Rüttimann *et al*., 1991).

## 2.8 Mixed culture

A group or colony of microorganisms present in a specific, localized location.

## 2.9 Anaerobic digestion

Anaerobic digestion is the process of decomposting of organic matter by a microbial consortium in an oxygen-free environment (Pain and Hepherd, 1985). It is designed to encourage the growth of anaerobic bacteria particularly the methane producing bacteria. This bacteria decrease the organic solids by reducing them to soluble substances and gases such as the methane and also the carbon dioxide.

The anaerobic digestion process is accomplished by four steps which are (1) hydrolysis of insoluble polymers by enzymes, (2) acidogenic fermentation with acetate is the main end product plus production of volatile fatty acids along with carbon dioxide and hydrogen, (3) acetogenesis which is the breakdown of volatile fatty acids to acetate and hydrogen and lastly, (4) methanognesis which is the conversion of acetate, formaldehyde, hydrogen and also carbon dioxide into methane and water.